Research Article

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Evaluation of the Microbiostatic Property of Newer Denture Disinfectant: An In Vitro Study

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ABSTRACT

Background: The purpose of this study is to evaluate and compare the efficacy of microbiostatic property of 2% Tea tree oil (TTO) with 1% sodium hypochlorite (NaOCl) on thermally activated polymethylmethacrylate acrylic resin plate exposed to Candida albicans, Staphylococcus aureus and Streptococcus mutans.

Materials and Methods: 60 of 30x10x2mm standardized acrylic resin plates of uniform surface were divided into 2 groups of 30 each for 2% TTO and 1% NaOCl. Ten from each group were colonised by 10^6 viable cells per millilitre of respective microorganisms and were disinfected for 10 minutes. The remnant adhered microorganisms were dispersed, diluted and plated onto culture media to determine the number of colony-forming units.

Result: 1% NaOCl showed complete retardation for all the organisms whereas 2% TTO was effective against streptococcus mutans. Isolated samples of Candida albicans, Staphylococcus aureus showed no retardation with 2% TTO.

Conclusion: The results suggest that 2% TTO is effective against all tested microorganisms.

Keywords: Acrylic Resin; Denture Disinfectant; Microbiostatic Property; Oral microflora, Sodium Hypochlorite; Tea Tree Oil.

INTRODUCTION

Surface irregularities or micro porosities of poly methyl methacrylate acrylic resin denture base greatly promote plaque accumulation by enhancing the surface area exposed to microbial colonization [1]. Dense microorganisms and their metabolites together form denture plaque. Plaque formed on the surfaces of removable dentures can have a significant impact on oral health, as well as it can lead to infection of denture-supporting mucosa (stomatitis), gum inflammation (gingivitis) and tooth decay.

When a patient visits a dental office to be treated with new dentures or to have an adjustment, repair or reline, the contaminated denture base may have to undergo a series of procedures during which microorganisms are transmitted through direct contact or through aerosol produced during trimming, finishing and polishing procedures. Opportunistic microorganisms with varying levels of pathogenicity of the denture plaque may spread and disseminate in the air leading to cross infection and exposing dental professionals and patients to various diseases [2].

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Daniela G Ribeiro et al [3] found that some of the species of oral pathogens, associated with denture plaque were Candida spp., Staphylococcus spp., Streptococcus spp. Therefore to obviate the cross infection from used dentures, as well as to help the patient to maintain normal oral hygiene while wearing the dentures, a need of using denture disinfectant would be obligatory.

Several chemical compounds in different concentrations are present in the market for the purpose of disinfecting denture bases. Literature on denture disinfection revealed that 1% solution of sodium hypochlorite as one of the most effective denture disinfectant [4]. However main drawbacks of sodium hypochlorite are skin irritation and destruction of clothes coming in contact [5]. Also soaking of prosthesis in sodium hypochlorite has shown to have deleterious effects on denture base materials, such as reduction of the flexural strength of acrylic resin and tarnish and corrosion of cobalt–chromium alloys. Additionally, the odour and aftertaste of this solution were not acceptable by many patients [6].

Many organic compounds with high degree of biocompatibility and few adverse reactions are being tried for human use, either as home remedies or for medicinal purposes [7]. One of the essential oils derived from *Melaleuca alternifolia*, tea tree oil (TTO) is native to the northeast coast of New South Wales. This Australian product is used in many parts of the world for its antimicrobial properties especially as a topical formulations [8]. In small concentrations, tea tree oil is useful for treating oral thrush, gingivitis, bad breath, tinea pedis, onychomycosis, healing acne, relieving irritation and itching from insect stings and bites and improving symptoms of genital herpes [9]. It is also known to be a root canal disinfectant in the endodontic practice [10]. In a range of concentration from 0.06 to 2%, TTO is known to be effective against many microbes [8].

From the above there is a reason to believe that it is a safe herbal derivative within prescribed concentrations for other dental applications. However there is no mention made in the literature of its use as denture disinfectant.

Within this context, considering the disadvantages of sodium hypochlorite and benefits of tea tree oil, a study is undertaken to evaluate and compare 2%TTO with 1% sodium hypochlorite for their microbiostatic property as denture disinfectant against Candida albicans, Staphylococcus aureus, and Streptococcus mutans.

MATERIALS AND METHODS

I) Preparation of acrylic resin (PMMA) plates to represent denture base:

IS 6887: 2005, Indian Standard for Denture Base Polymers had not mentioned dimensions for PMMA specimen strips to be tested for microbicidal property of reagents on denture base resins. Therefore sixty 30x10x2mm acrylic plates were obtained using thermally activated acrylic resin(Denture Base Polymer Resin (Heat cure) DPI Dental Products Of India Bombay Burmah Trading Co Ltd, India) to suit the diameter of 6 well culture plate (Corning® Costar® cell culture plates 6 well, flat bottom) used in this study. These plates were fabricated from standardized plaster moulds. The moulds were obtained using acrylic resin strips of 30x10x2mm dimensions cut through laser from commercially available acrylic resin sheet having thickness of 2mm X 200mm X 150mm.The obtained thermally activated acrylic resin plates were trimmed, polished and measured to the size using Vernier calliper and were stored in distilled water for 3days with water being changed 24hourly. They were divided into 2 groups of 30 each, one group for 1% sodium hypochlorite (group 'S') and the other for 2% TTO (group 'T'). Each of these groups was further subdivided into 10 plates each for 3 specific organisms under study. They were grouped as SCA, SSA, and SSM for Sodium hypochlorite Candida albicans, Sodium hypochlorite Staphylococcus aureus, Sodium hypochlorite Streptococcus mutans and TCA, TSA, TSM for Tea tree oil Candida albicans, Tea tree oil Staphylococcus aureus, Tea tree oil Streptococcus mutans respectively.

II) Preparation of disinfectant solutions

Tea tree oil is available in market in 100% concentration. To make it as proposed denture disinfectant in this study, it is required to be diluted to 2% solution. "Scientific Committee on Consumer Products (SCCP) Opinion on Tea Tree Oil" suggested that tea tree oil is insoluble in water, but miscible with ethanol[11]. Ethanol is a versatile

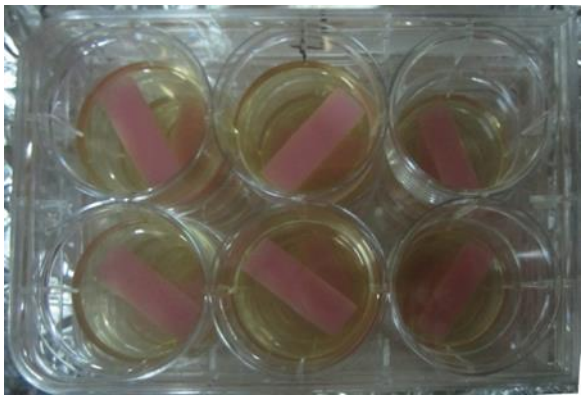

Fig 1: Specific standardized strain suspension with 1.5 ml of respective medium and one acrylic specimen.

Fig 2: Complete retardation of colonised acrylic resin plates when subjected to 1% sodium hypochlorite for 10 minutes.

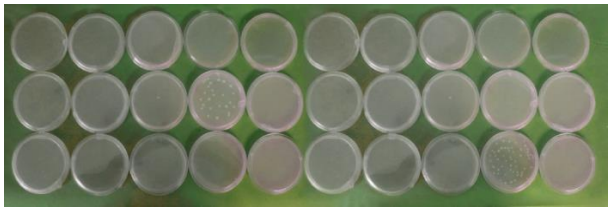

Fig 3: Isolated samples showed no retardation in respect of Candida albicans and Staphylococcus aureus.

solvent, miscible with water and with many organic solvents. SO, 2% tea tree oil disinfectant solution was prepared by adding 0.5ml of 100% Tea Tree Oil (Health post company, New Zealand.), 5ml of absolute ethanol (Changshu yangyuan chemical,China. Lot no XK-13-201- 00185) and 19.5 ml distilled water making a total of 25ml. Four such preparations were made to obtain 100ml of solution. Similarly 1% of sodium hypochlorite disinfection solution was made by adding 10ml of 5% sodium hypochlorite (5% - Rankem. Product code S0861) to 40 ml of distilled water. Two such preparations were made to obtain 100ml of solution.

III) Preparation of specimens (colonized acrylic resin plates with selected micro organisms)

On the day of experiment, acrylic resin plates were washed in distilled water and transferred to the sterilized bijou bottles containing 0.85% sodium chloride (S0817 SIGMA-ALDRICH) and subjected to autoclaving at121 degree C, under15 lbs for 15 minutes.

Table 1: Describes the growth of microorganisms on culture plates after the specimens were subjected to 1 % sodium hypochlorite. 0 dilution, 10 times dilution, and 100 times dilution denote the strength of physiological solution with detached microorganisms.

Effect Of 1% Sodium Hypochlorite Disinfectant Solution On Colonised Plates				
Organisms	Notation and No. of plates	Quantity of growth		
		0 dilution	10 times dilution	100 times dilution
Candida albicans	SCA- 10	No growth	No growth	No growth
Staphylococcus aureus	SSA- 10	No growth	No growth	No growth
Streptococcus mutans	SSM- 10	No growth	No growth	No growth

Table 2: Complete retardation of colonised acrylic resin plates of Streptococcus mutans when subjected to 2% TTO for 10 minutes.

Notation	cfu/ml at 0 dilution	cfu/ml at 10 times dilution	cfu/ml at 100 times dilution
TSM 1	-	-	-
TSM 2	-	-	-
TSM 3	-	-	-
TSM 4	-	-	-
TSM 5	-	-	-
TSM 6	-	-	-
TSM 7	-	-	-
TSM 8	-	-	-
TSM 9	-	-	-
TSAM10	-	-	-

Table 3: Isolated samples showed no retardation in respect of Candida albicans when subjected to 2% TTO.

Notation	cfu/ml at 0 dilution	cfu/ml at 10 times dilution	cfu/ml at 100 times dilution
TCA 1	-	-	-
TCA 2	-	-	-
TCA 3	-	-	-
TCA 4	-	-	-
TCA 5	68 X10^1	33 x 10^2	3 x 10^3
TCA 6	-	-	-
TCA 7	-	-	-
TCA 8	-	-	-
TCA 9	-	-	-
TCA 10	-	-	-

Table 4: Isolated samples showed no retardation in respect of Staphylococcus aureus when subjected to 2% TTO.

Notation	cfu/ml at 0 dilution	cfu/ml at 10 times dilution	cfu/ml at 100 times dilution
TSA 1	109 X10^1	20 x 10^2	3 x 10^3
TSA 2	-	-	-
TSA 3	-	-	-
TSA 4	-	-	-
TSA 5	-	-	-
TSA 6	-	-	-
TSA 7	-	-	-
TSA 8	-	-	-
TSA 9	-	-	-
TSA 10	-	-	-

A working broth was prepared by suspending 30.00 grams of Sabouraud's dextrose broth (HIMEDIA M033), 13 grams of nutrient Broth (HIMEDIA M002), 37grams of Brain Heart Infusion broth (HIMEDIA M211) in 1000 ml distilled water(as per the directions of HIMEDIA company) individually and was autoclaved at 121 degree C, under15 lbs pressure for 15 minutes. Later Candida albicans- MTCC 227, Staphylococcus aureus- MTCC 6809, Streptococcus mutans- MTCC 497 were added to their respective working broth and inoculated for 48 hrs at 37^0c.

After this period, acrylic resin plates of 20 each were colonized with respective microorganisms by a suspension containing 10^6 viable cells per millilitre. The suspension was prepared in sterile saline solution (0.85% NaCl) with the aid of BioTek multi mode detector (synergy HT – Multi mode micro plate reader, BioTek), adopting the wavelength of 530 nm and optical density of 0.284 for Candida albicans[12], wavelength of 490 nm and optical density of 0.374 for Staphylococcus aureus [4] , the wavelength of 398 nm and optical density of 0.620 for Streptococcus mutans [4].

Adherence testing was performed in an aseptic environment, in a laminar air flow chamber using 6-wells plates (Corning® Costar® cell culture plates 6 well, flat bottom). For this purpose, 0.1 ml of specific standardized strain suspension with 1.5 ml of respective medium and one acrylic specimen are placed in each plate. (Fig 1) The plates are sealed and incubated at 37°C for 24 hours[12].

10 specimens from each of the respective group were subjected to 1%sodium hypochlorite and the other 10 to 2% tea tree oil for 10 minutes each. After immersion, the specimens were removed, washed with 2 ml of sterile distilled water and transferred to tubes containing 1 ml of sterile physiological solution (0.85% NaCl) and glass pearls. The tubes were agitated in a Vortex stirrer for 60 seconds and the adhered cells were dispersed. This initial suspension was diluted by 10 and 100 times in physiological solution, and 0.1 ml of each suspension was spread on corresponding culture plate. After 48 hours of incubation at 37°C, the number of colony-forming units per specimen (cfu/ specimen) was determined and tabulated[12].

Because the newer compound was investigated for its microbiostatic property for clinical use, uncompromised protocol was observed while handling the microorganisms and solutions being prepared thereof. Following results were recorded.

RESULTS

Colonised acrylic resin plates subjected to 1% sodium hypochlorite for 10 minutes showed complete retardation (Table 1) (Fig 2). 2% tea tree oil as a denture disinfectant was effective against streptococcus mutans completely (Table 2). Isolated samples showed no retardation in respect of

Candida albicans and Staphylococcus aureus (Table 3 and Table 4) (Fig 3).

DISCUSSION

It is mandatory that dentures should be disinfected before transported to laboratories for fabrication as well as before delivering to the patient. Likewise they require disinfection in the dental clinic as well as in laboratory to eliminate cross contamination [13]. The contamination usually occurs when micro-organisms spread by direct contact with blood or saliva from an infected patient in the clinical area or by indirect contact with micro-organisms from dental prosthesis in the clinical and laboratory areas [14]. Candida spp., Staphylococcus spp., Streptococcus spp., are predominant micro flora usually associated with denture plaque and Candida albicans is the most frequently isolated fungal species [3].

When the immersion procedure is used, the choice of the disinfectant should be made with regard to its effectiveness in inactivating microorganisms without any adverse effects on the denture materials [13]. Researches on denture disinfection revealed that full-strength sodium hypochlorite when used has shown to be effective against microorganisms including spores. Senia et al [15] have shown that l-minute soak in 5.25% sodium hypochlorite (undiluted Clorox) sterilized gutta-percha cones used for filling root canals. Trevelyan [16] had shown that sodium hypochlorite was effective against hepatitis virus. Abbott and Trevenol laboratories recommended 2.5% sodium hypochlorite for inactivating liquid wastes containing the infectious agent of viral hepatitis [17]. Katherine R. Carson had shown that 6% sodium hypochlorite as root canal irrigant was effective against Lactobacillus spp [18]. In an in vitro study Francine Cristina da Silva had shown that acrylic resin specimens, contaminated with prevalent organisms like Candida albicans, Streptococcus mutans, Staphylococcus aureus when subjected to 1% sodium hypochlorite for 10 minutes duration, growth of organisms was completely retarded [4].

However sodium hypochlorite has certain disadvantages, such as unpleasant odour and the possibility of causing acrylic resin bleaching and a corrosive effect on metal components, such as cobalt-chrome structures for partial removable dentures. It depends on the concentration of the disinfection and period of immersion of the prosthesis [10].

Since synthetic chemical products always showed certain disadvantages, a study was under taken to investigate the possibility of denture disinfectant based on herbal extracts. Some of the earlier researches revealed that Australian tea tree oil, peppermint oil, sage oil and manuka oil proved to be the most potent essential oils against oral pathogens [19, 20].

In the present study, 2% tea tree oil was selected to evaluate and compare its microbiostatic properties with those of a commercially popular denture disinfectant (1 % sodium hypochlorite) on predominant microflora of denture biofilm because of its wide action of anti microbial properties [8].

Tea tree oil in a concentration of 2% was selected based on the susceptibility data available for bacteria and fungi tested against M. alternifolia oil [8].

Microbiostatic action of the two disinfectants

In this study, 1% sodium hypochlorite solution was noticeably effective against Candida albicans, Staphylococcus aureus, and Streptococcus mutans, when subjected for 10 minutes immersion. These results were in accordance with the studies of Francine Cristina da Silva [4], Katherine R. Carson [18], The efficacy of lower concentrations of sodium hypochlorite in this study could be attributed because of wide area of exposure.

Similarly 2% tea tree oil as a disinfectant was noticeably effective against lactobacilli and streptococcus mutans. However plating of serial dilution of physiologic solution with dispersed cells pertaining to one out of ten acrylic resin plates of candida albicans and Staphylococcus aureus each, showed colony forming units on respective culture plates. Reasons for growth could be that acrylic resin plates used in this study were standardized dimensionally using Vernier calliper, whereas visually examined for their surface uniformity. Any pear shaped subsurface porosity or void within the acrylic resin plate would have allowed the growth in to the defect, but might not have allowed the

percolation of disinfectant. So those unexposed cells would have resulted in the formation of colonies on culture plates. This study showed the possibility of tea tree oil at 2% concentration as an effective denture disinfectant against Staphylococcus aureus, Streptococcus mutans, and Candida albicans at 10 minutes' immersion.

Limitations of this study included that only 3 species of the microorganisms of denture biofilm were studied and experimental denture disinfectant was studied for its microbiostatic properties only. Its effect on mechanical, physical and chemical properties of denture base resin need to be studied further. This study was conducted with the experimental denture disinfectant of one concentration only. Further studies with multiple concentrations and different exposure times may provide more conclusive observations.

CONCLUSION

From the results obtained in this study, it was concluded that, among the disinfecting solutions tested

1% sodium hypochlorite disinfectant was found to be effective against Candida albicans, Staphylococcus aureus, and Streptococcus mutans at10 minutes immersion

2% tea tree oil disinfection was found to be effective against Candida albicans, Staphylococcus aureus, and Streptococcus mutans at 10 minutes immersion.

CONFLICT OF INTEREST

No potential academic conflict of interest relevant to the above article was reported.

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